SIMULATION OF FOREST ENVIRONMENT AS A MEASURE TO SUSTAIN PROTEIN AND VITAMIN CONTENTS IN DOMESTICATED SPECIES OF WILD VEGETABLES

Kimambo, M.W., Mwamakimbullah, R.J.L. and Hamza, K.F.S.

Sokoine University of Agriculture
Faculty of Forestry and Nature Conservation
Department of Wood Utilisation Box 3014, SUA
Morogoro

ABSTRACT

The most suiting types of shade under which domesticated wild Vegetables could be grown to sustain their nutritive values were determined. Seeds from *S. scabrum, L. cornuta, B pilosa* and *B. alba,* the most preferred wild Vegetables in Lushoto district, Tanga region in Tanzania were collected, managed and then sown in nurseries covered by different types of shades (i.e. under banana plantations, under improvised shade, in open field, under tree covers). Wild Vegetables in their natural environment were used as a control of the experiment. Leaves from domesticated and wild Vegetables were harvested and protein, vitamin A (ß-carotene) and C content determined. It was observed that domestication and shade types under which of wild Vegetables were grown had effects on their nutritive values (the protein, vitamin A and C contents). Nevertheless, banana shades closely upheld vitamin C contents of *L.curnuta* and *B. pilosa*. Also, it did well to maintain protein contents in *L. curnuta* and *S.scabrum* as well as Vitamin A contents in *B.alba.* On the other hand, tree shades were good in sustaining Vitamin A and protein contents in *S.scabrum* and *B.alba,* respectively. Growing *S. scabrum, L. curnuta* and *B. pilosa* in the open helped to closely retain Vitamin C, Vitamin A and Vitamin A, respectively. Improvised shades were of little help in sustaining nutrient contents in all vegetables.

INTRODUCTION

Wild Vegetables are non-timber forest products whose leaves or aerial parts have been integrated in a community's culture for use as food or traditional medicine. They may be sourced from the wild (in nature) or may be domesticated in house gardens and farm lands (Ogoye-Ndegwa and Aagaard-Hansen 2003; Midmore *et al.* 1991. Wild Vegetables support human life of the communities in different ways. They give diversity, and add flavour and taste to daily food intake (Asfaw 1997). Furthermore, they may have a higher nutrition value than some of the introduced foreign Vegetable varieties such as cabbage, lettuce and spinach (Weinberger and Msuya 2004; Ezekwe *et al*. 1999; Mathenge 1997; Nordeide *et a*l. 1996; Humphry *et al.* 1993). Wild Vegetables are an important source of carbohydrates, proteins, fibre, vitamins (such as vitamins A and C), and minerals (such as zinc, iron, calcium) and iodine (Ohiokpehai 2003, Kinabo *et al.* 2004). In addition, wild Vegetables are also important in their ecological, agronomic, cultural values; and creation of employment (Abbiw 1997; Okafor, 1997). Besides being used as food, some of the Vegetables are known to be of medicinal value (Olsen and Nielsen, 1999; UNESCO, 1997). They are used to treat different kinds of ailments, ranging from simple wounds to more complex ailments. Ecologically, wild leafy Vegetables have a unique advantage within the local farming systems in that they thrive during the dry and wet season and many of them are environment friendly hence require no pesticides and fertilizers in their management (Chweya and Eyzaguirre 1999; UNESCO 1999).

Micronutrient deficiency is a universal problem, which presently affects over two billion people worldwide, resulting in poor health, low worker productivity, and high rates of mortality and morbidity (Flyman and Afolayan 2006). Deficiency in micronutrients has led to increased rates of chronic diseases and permanent impairment of cognitive abilities in infants born to micronutrient deficient mothers. Wild Vegetables have been the mainstay of human diets for centuries, providing millions of consumers with important micronutrients, such as vitamins and minerals needed to maintain health and promote immunity against infections. According to Humphry *et al.* (1993), several studies show that many species of wild green Vegetables are rich sources of nutrients. Hence, they could make an important contribution to combating micronutrient malnutrition providing nutrient as well as food security. Unfortunately, wild Vegetables are currently underutilized, and have been neglected by researchers. Their promotion and integration into human diets could assist in their protracted use and conservation. However, if their extensive uses were to be achieved, there is a high likelihood that depletion of the wild Vegetables from their natural habitat will occur. Therefore, to sustain them, there is a need to preserve and exploit them in a planned manner. This can be achieved through their domestication so that their performance is studied and managed just like other crops. Normally, domestication of wild Vegetables subjects them to different environmental conditions, in terms of soil type and composition, humidity and other factors which might change the plant physiology. Studies by Edmonds and James (1997) and (Michele *et al.* 2007) have indicated that domestication of wild Vegetables have resulted in them altering their nutritive values. Therefore, domestication efforts of wild Vegetables should go hand in hand with those to determine optimal growing conditions in which domesticated wild Vegetable could maintain their nutritive values. This study aimed at simulating natural growing conditions of wild Vegetables' by using different types of shades to determine a shade(s) which maintained their nutritive value. Protein and Vitamins A and C were of interest to this study. Therefore, the study determined contents of vitamin A, vitamin C, protein and moisture content in the community's most preferred domesticated wild Vegetables and compared to their counter parts in the natural growing condition.

A study by Bowman and Russel (2001) indicated that active forms of vitamin A participate in three essential functions, namely, visual perception, cellular differentiation and immune response, while vitamin C plays the role of a biological reducing agent (antioxidant) and may be linked to its prevention of degenerative diseases, such as cataracts, certain cancers and cardiovascular diseases. On the other hand, proteins are emphasized in diets of young children as their deficiency may cause kwashiorkor, which is very common in weaned children. The deficiency of both carbohydrates and protein causes underweight in children, which could affect the growth of children causing brain under development (Chweya 1994).

MATERIALS AND METHODS

Study Area Description

The study was conducted in two villages namely; Kweminyasa and Mahange which surround the Balangai Forest Reserve in Lushoto district, Tanga region in Tanzania. In this area, annual temperature varies with altitude, where at 500 meters above sea level, temperature ranges from 25 to 27^{o}C while between altitude 1500 to 1800 above sea level, temperatures range from 16 to 18^{o}C. The area has bimodal rainfall pattern which ranges between 600 to 1200 mm. The dominating soil type is latosols, however, in the lower wetter areas soils are humic ferralic whereas in the drier area soils are humic ferrisol.

2.2 Sampling of households

In collaboration with farmers and with the assistance of the village extension officers, two study villages were selected. This was followed by interviews to households to identify types of wild Vegetables which are most preferred and commonly consumed by people in the study community. The sample of households interviewed was determined using expression (Kothari, 2004):

$$n=\frac{Z^2pq}{e^2}$$

Where,

n = desired sample size;
z = the value of the standard variate at 95 per cent confidence level
p= sample proportion of the target population (users of wild Vegetables), q = 1 – p.
e = the acceptable error.
p = was determined through a pilot survey and
e = was determined iteratively on site.

Thereafter, a self administered questionnaire was used to capture information about preferred wild Vegetables by households. Preference was assessed by respondents/households (represented by one family member) giving scores to each of the listed wild Vegetables using Likert scale. The most preferred wild Vegetables scored 4, followed by 3, 2, 1 and 0. In that order, the least preferred scored 1 and those not preferred at all scored 0.

Dry seeds from most preferred vegetables were harvested, pooled and stored in a paper bag. No further treatment was applied to the seeds. They were planted directly after one week storage at room temperature in a dry room. Before sowing, seed beds were prepared by simple ploughing, just as it is done by farmers in the area. No fertilizer was added. Where, vines were used, vines of about 15 to 20 cm in length were cut and planted directly without storage. Seeds and vines were sown within the habitats of communities at Kweminyasa and Mahange villages under four different growing conditions, namely: tree shades, banana plantations, improvised shade and open spaces. The plants were provided with supplementary irrigation using watering cans, which is a common practice in the area. Encroaching plants such as weeds were hand-picked to remain with clear stands of intended plants in each respective plot.

Experimental design

A Randomized Complete Block Design (RCBD) was used in this study. Plots under different shades (blocks), namely, garden under banana plantations, garden under improvised shade (i.e. grass shade supported by a couple of poles), garden in open field and garden under tree cover, received four treatments (Vegetable species) and each plot replicated three times. Wild Vegetables in their natural environment were used as a control of the experiment.

Field procedure

Samples of leaves (from domesticated and wild Vegetables) for laboratory analysis were harvested when plants were of one and half months old. In order to determine nutrient levels of beta-carotene, Vitamin C and proteins in leaves of Vegetables, samples were taken before the onset of fruiting to determine their levels at this developmental stage. The edible portion, which included leaves and young petioles were cut as practiced by the Balangai communities when they harvest wild Vegetables. Care was taken to only pick plant's parts and not any other material that would affect laboratory results e.g. insects, twigs or other plant materials (i.e., extraneous matter). The picked parts were briefly air swilled (shaken) to remove dust and soil particles. After harvest they were packed in air tight bags. Care was taken not to further expose the samples to sunlight after harvesting by undertaking these last steps in the shade and immediately storing them in a cool box with ice packs to maintain a temperature of + 4 to 8°C. On the way to Sokoine University of Agriculture laboratories, the cool boxes were replenished with fresh ice every 12 hours. Collection of leaves from wild Vegetables in natural environment was done at around half a kilometre to two kilometres into the forest to avoid boarder effects on the vegetables.

Determination of moisture content
Moisture content was determined using AOAC (1995) by drying a known sample weight in an oven at 105ºC for 24 hours until constant weight was obtained. The difference between sample weight and oven dry weight of the sample is termed as moisture content of the sample.

Determination of the protein content
Crude protein was determined by the micro- Kjeldahl method (AOAC, 1995). A 5g of dried samples were weighed into a tarred filter paper and put into 100ml Kjeldahl digestion tubes. A blank was prepared as a control of the experiment by dropping a piece of a filter paper without sample into the 100 ml digestion tube. To each test tube 2g Kjeldahl catalyst (Copper sulphate) and 5ml of concentrated sulphuric acid was added. The sample was digested until a clear blue solution was obtained. The digested sample was cooled and then 20ml of distilled water was added to dissolve the content. The diluted digest was distilled using a micro distillation apparatus. A 20ml of 45% NaoH solution was added to the digest to facilitate release of ammonia. The ammonia was extracted by steam distillation and collected in 5ml of 4% Boric acid. 35ml of ammonia distillate was titrated with 0.02N HCl standard solution using Bromocresol green, methyl red mixture as indicator. The amount of solvent used reflects nitrogen content of the sample. Therefore, Nitrogen % was calculated using the following expression:

$$N\% = \frac{(\text{Titre-blank})\text{in ml}*0.014077*100}{\text{Weight of the sample (g)}}$$

In order to attain protein content of the sample, the calculated N% was multiplied by a common factor of 6.25.

Determination of ß-carotene (vitamin A)
The ß-carotene content was determined by using the high-performance liquid chromatography (HPLC) method as described by Kimura and Rodriguez-Amaya (2003). One gram of vacuum-dried Vegetable was mixed thoroughly with 100 ml of hexane: acetone (6:4, v/v) solution, and 0.1 g $MgCO_3$ in a homogenizer. Acetone was then washed out five times with salt-saturated water. The hexane extract was filtered with a 0.45-µmol filter. Analyses were performed using HPLC (HPLC; SPD-10AV SHIMADZU) equipped with a 717 plus auto sampler, 600 controller, 996 photodiode array detector with a 125 × 4-mm LiChrospher 100 RP-18e column, 5 µm (Merck, Darmstadt, Germany) under isocratic conditions at ambient temperatures. The mobile phase was acetonitrile: methanols (75:25, v/v) at a flow rate of 1.5 mL/min. ß-carotene quantification were carried out at a single wavelength of 436 nm. Concentration of purified ß-carotenoid standard was determined using the tabulated absorption coefficient of $A_{1\,cm}^{1\%}$ (i.e. absorbance at a given wavelength of a 1% solution in 1 cm light-path spectrometer cuvette) (Britton 1995).

Determination of vitamin C
Initially, the Ascorbic acid content of the samples were estimated by macerating the samples mechanically with a stabilizing agent Trichloroacetic acid and the filtered extract was titrated with 2-6-Dichlorophenolindophenol (AOAC method 1995). 109 samples (Vegetable pulp) were weighed in a beaker and transferred into a mortar and ground with acid washed sand and TCA solution. Then the ground sample was transferred quantitatively into a

volumetric flask (100 ml capacity), and made to volume using TCA solution. Then, the mixture was mixed well and filtered using whatman filter paper No.l. Ten mls of each sample were taken into the 200ml conical flask, and the samples were then titrated with indophenol solution until a pale pink color appeared. A blank was also determined. Ten mls of TCA solution was put into the flask, then distilled water of the same volume as that of standard indophenol solution was added. The solution was also titrated with indophenol solution until a pale pink color appeared. Thereafter, the vitamin C was determined using the following expression:

$$\text{Vitamin C content}\left(\frac{\text{mg}}{100\text{g}}\right)=\frac{(A-B)*C*100*100}{10*S}$$

Where:

- C= mass in mg of ascorbic acid equivalent to 1.0 of standard indophenol solution.
- S= weight of sample taken, gm
- A= volume in ml of the indophenol solution used for sample.
- B= volume in ml of the indophenol solution used for blank

Data Analysis

Data were analysed using GLM procedures of SAS (2001) with shades (control, improvised, trees, banana shade and open field) and species (independent variables against nutrient concentration (vitamins and proteins,). Residues were considered as random effects and that means were separated by the Least Squares Means separation test using the PDIFF option when the respective F-test was significant ($P < 0.05$).

RESULTS AND DISCUSSION

A total of 30 households' representatives participated in this study. They indicated that the most preferred wild vegetables were *L. cornuta, B. pilosa, B. Alba* and *S. scabrum.*

Effect of shade type on moisture content (mg/100g)

Moisture contents of the studied Vegetable species in natural environment and when domesticated is presented in Table 1. As seen from the Table, in terms of moisture contents, there were no significant differences among species and growing conditions.

Table 1: Effect of shade on moisture content (mg/100g) for four species of wild Vegetables

Vegetable species	Shading					Significance
	Forest	Improvised	Tree	Banana	Open Space	
L. cornuta	87.2	85.2	86.0	86.9	87.2	NS
B. pilosa	84.3	84.6	85.6	85.2	82.6	NS
B. alba	90.5	89.4	91.2	81.6	89.4	NS
S. scabrum	81.7	88.0	89.6	89.9	82.8	NS
Significance	NS	NS	NS	NS	NS	

Key:

***Within rows:** least squares means with common superscript are not significantly different ($P>0.05$).

Significance: NS, not significant; *, $P<0.05$; **, $P<0.01$;***, $P<0.001$

Effect of type of shade on vitamin C content (mg/100g)

Results presented in Table 2 present Vitamin C contents in leaves of wild Vegetables in the natural environment (control) and domesticated ones under different types of shades. It is vivid that in their natural environment, each Vegetable has its own levels of vitamin C led by *S. scabrum*.

Table 2: Effect of type of shade on vitamin C content (mg/100g)

Vegetable species	Shading				
	Control (Forest)	Improvised	Tree	Banana	Open Space
L cornuta	0.7[d]	2.3[b]	3.1[a]	1.2[c]	1.2[c]
B. pilosa	1.7[c]	1.1[d]	2.4[b]	1.8[c]	2.8[a]
B.alba	0.8[c]	1.2[b]	1.3[b]	1.5[b]	1.6[a]
S. scabrum	3.4[a]	1.7[c]	1.6[c]	1.3[c]	2.5[b]
Significance	***	***	***	NS	***

Key:
***Within rows:** least squares means with common superscript are not significantly different (P>0.05).
Significance: NS, not significant; *, P <0.05; **, P <0.01;***, P <0.001

When domesticated, *L. cornuta* is very sensitive to the type of shade under which it is grown. When grown in the open or under banana trees shades, it provides vitamin C value of 1.2 (mg/100g) compared to 0.7(mg/100g) provided under control or forest cover. The two shades simulate the forest condition relatively better than others. Limo *et al*. (2003) in a study carried out in Morogoro, Tanzania, reported vitamin C content in *L. cornuta* to be 15.9 mg/100g. Indeed, the two figures differ considerably and this could be attributed to the differences in soil fertility and localities from which the wild Vegetables were obtained.

From this study, however, interesting result was noted from *L. cornuta* which appears to be capable to produce higher levels of vitamin C, given it is planted under shades of trees.

Regarding *B. pilosa,* a shade provided by banana trees simulated better the forest conditions (the control). There was no significant difference between Vitamin C contents achieved under the two shades. Therefore, planting *B. pilosa* under banana trees will be the most ideal place if one wants to maintain vitamin C in this Vegetable.

B. alba, like *L. cornuta,* was very sensitive to the type of shade under which it was grown. It was unclear as to why none of the shades could successfully simulate the forest conditions for it to sustain vitamin and protein contents as while in the forest. All types of shades increased vitamin C content level when compared to the control scenario. Improvised, banana and tree shades although had different vitamin C contents but the differences were not significant. Planting *B. Alba* under any of the shades won't change its vitamin C content significantly since all of them simulated better the forest conditions compared to open space.

The results also indicated that domestication of *S. scabrum* under the studied shades significantly reduced its vitamin C contents. Therefore, *S. scabrum* should be planted in the open since in that environment produced better results closer forest levels.

Effect of type of shade on vitamin A content

Results presented in Table 3 present Vitamin A contents in leaves of wild Vegetables in the natural environment (control) and domesticated ones under different types of shades. In natural environment, it is vivid that in their natural environment each Vegetable had its own levels of vitamin A but *S. scabrum* had the highest amounts.

Table 3: Effect of type of shade on vitamin A µg/g

Vegetable species	Shading				
	Forest	Improvised	Tree	Banana	Open Space
L.cornuta	225.1^{e}	326.0^{c}	480.6^{a}	467.7^{b}	287.4^{d}
B.pilosa	236.4^{b}	349.1^{c}	392.0^{a}	397.0^{a}	237.5^{b}
B. alba	250.5^{d}	297.3^{b}	339.3^{a}	279.6^{c}	185.5^{e}
S. scabrrum	455.7^{a}	226.3^{d}	234.9^{c}	218.8^{e}	291.2^{b}
Significance	***	***	***	***	***

Key:

***Within rows:** least squares means with common superscript are not significantly different (P>0.05).

Significance: NS, not significant; *, P <0.05; **, P <0.01;***, P <0.001

The results show that domestication of *L. cornuta* under any of study's shades increases the amount of vitamin A. If anything, *L. cornuta* should be planted in the open since in that environment produces better results closer to forest levels. Open space again simulated better the forest conditions in vitamin A contents in *B. pilosa*. There was no significant difference in vitamin A contents between what *B. pilosa* produced in the forest and in the open. Except when *B. alba* is grown in the open, all other conditions lead to significant increase in vitamin A contents. Planting *B. alba* under shades of banana produced results closer to those of the forest.

In addition, results in Table 3 show that simulation of the growing condition for *S. scabrrum* in regards to vitamin A was not successful. All types of shades produced almost half of what it naturally contains in the forest. This suggests that unless more efforts are directed towards determination of better growing condition, the forest could be the only better place for *S. scabrrum*. Nevertheless, the amounts of *S. scabrrum* found under the improvised, tree and banana shades, and in the open at this study site were higher than 100 and 75.4 mg/g reported by Mwajumwa *et al.* (1991) and Weinberger and Msuya (2004), respectively. Since the two study sites were different, locational difference may have contributed to the observed variation in *S. scabrrum* contents.

Effect of Type of Shade on Protein Content

Results presented in Table 4 present protein contents in leaves of wild Vegetables in the natural environment (control) and domesticated ones under different types of shades. It is vivid that when grown in their natural environment each Vegetable has its own levels of protein contents. *S. scabrum* commands the highest amounts.

Table 4: Effect of type of shade on crude protein content (mg/100g)*

Vegetable species	Shading				
	Forest	Improvised	Tree	Banana	Open Space
L. cornuta	0.4^{d}	1.2^{c}	1.6^{a}	0.3^{d}	1.4^{b}
B. pilosa	0.4^{d}	2.4^{c}	3.3^{b}	6.3^{a}	6.5^{a}
B. alba	0.4^{d}	1.6^{c}	0.2^{e}	2.9^{b}	3.3^{a}
S. scabrum	6.4^{a}	1.8^{c}	1.8^{c}	3.3^{b}	1.5^{d}
Significance	***	***	***	***	***

Key:

Within rows: least squares means with common superscript are not significantly different (P>0.05).

Significance: NS, not significant; *, P <0.05; **, P <0.01;***, P <0.001

A study by Chweya (1994) observed a protein level ranging from 0.8 to 5.5 g/100 g in fresh leaves of nine species of traditional leafy Vegetables commonly eaten in Kenya. In this study, except for *S. scabrum* other Vegetables compare less to their Kenyan counterparts. This could be explained by the fact that some species are habitat specific (Maundu *et al*. 1999).

Furthermore, the results in Table 4 indicated that for *B. pilosa, B.alba* and *S.scabrum* the study failed to capture ideal conditions for them in the homestead environment to retain their protein contents. The applied shades to *B. pilosa* and *B. alba* increased the protein content while in *S. scabrum* the contents decreased. Domestication by growing L. *cornuta* under banana tree almost maintained its protein content (P>0.05).

CONCLUSION AND RECOMMENDATION

Conclusion

The type of shades under which domesticated wild Vegetables were grown had an effect on the nutritive values of these Vegetables. However, natural growing conditions were well simulated for *L.curnuta, B. pilosa and B. alba* by the different shades presented in the study. However, for unknown reasons, *S. scabrum* proved to be very difficult to domesticate.

Recommendation

Based on the findings from this study, Table 5 presents the best growing condition under which domesticated wild Vegetables sustain or improve their nutritive values. Depending on the domestication purpose, for example, if the purpose is to at least sustain the quality of wild Vegetables, then the domestication of wild Vegetables should be done under better simulating shades (column 4). However, if the purpose is to increase the nutritive contents, then domestication could be done under shades that provide the highest values (column 5).

Table 5: Recommended shade types for sustaining vitamin and protein levels in domesticated wild Vegetables

Nutrient	Species name	Nutrient value in forest	Better simulating Shade and its nutrient value in bracket	A shade with the highest nutrient content and its nutrient value in bracket
Vitamin C (mg/100g)	*L. curnuta*	0.7	Banana (1.2)	Tree (3.1)
	B.pilosa	1.7	Banana (1.8)	Open space (2.8)
	B. alba	0.8	Improvised shade (1.2)	Open space (1.6)
	S. scabrum	3.4	Open space (2.5)	Open space (2.5)
Vitamin A (µg/100g)	*L. curnuta*	225.1	Open space (287.4)	Tree (480.6)
	B.pilosa	236.4	Open space (237.5)	Banana (397.0)
	B. alba	250.5	Banana (279.6)	Tree (339.3)
	S. scabrum	455.7	Tree (234.9	Tree (234.9)
Protein (mg/100g)	*L. curnuta*	0.4	Banana (0.3)	Tree (1.6)
	B.pilosa	0.4	Improvised (2.4)	In the open (6.5)
	B. alba	0.4	Tree (0.2)	In the open (3.3)
	S. scabrum	6.4	Banana (3.3)	Banana (3.3)

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